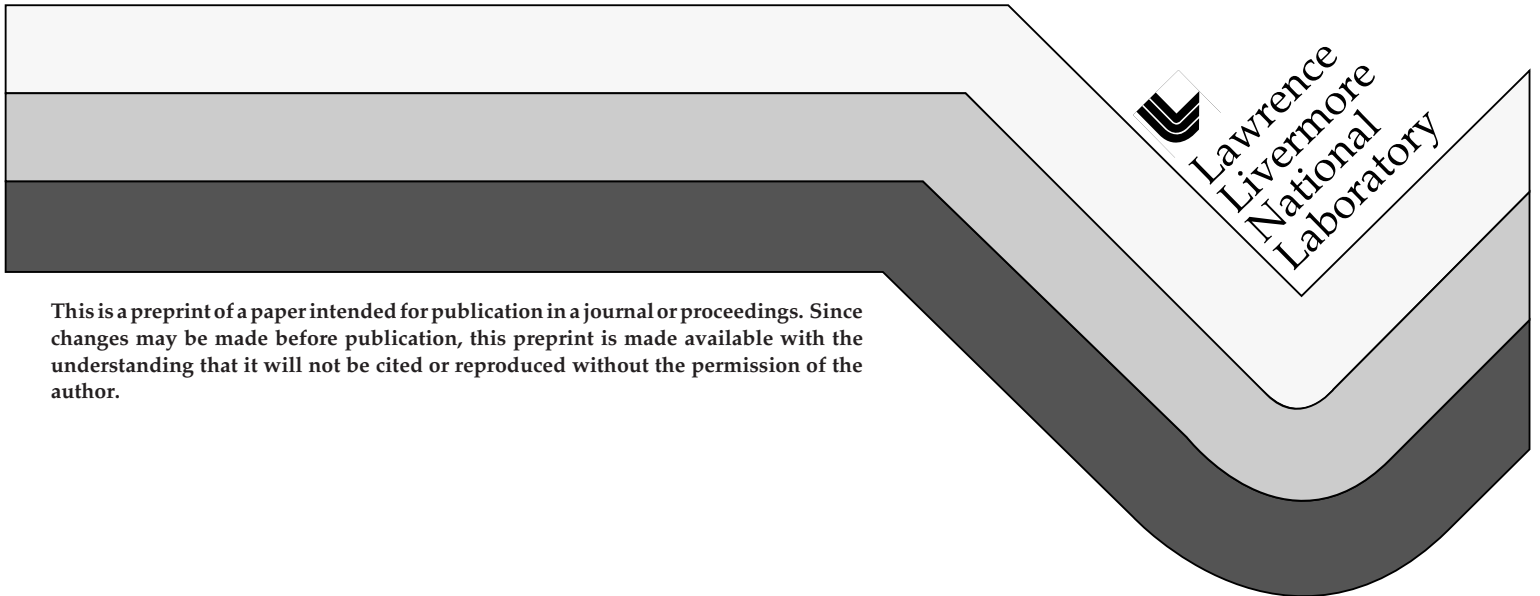


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Subsurface imaging using the Spectral Polarization Difference technique and NIR illumination

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ABSTRACT

A subsurface imaging system is utilized to test the ability of the spectral polarization difference imaging technique for deep subsurface imaging in tissues. The illumination of the system is derived from compact class III lasers in the red and NIR spectral region and, alternatively, from a white light source and selection of the appropriate illumination wavelength using band-pass optical filters. The experimental results demonstrate detection and imaging of a high-scattering object located up to 1.5 cm underneath the surface of a host chicken tissue.

Key words: Polarization, tissues, light scattering, imaging.

1. INTRODUCTION

Photonic technology is currently playing an important role in the development of new medical diagnostic tools. The widespread availability of solid state near infrared laser sources providing illumination that can penetrate the tissue at a larger depth has been crucial for the advancement of these cutting edge, less or non invasive optical approaches for medical diagnostics. Optical imaging and optical biopsy are two of the research areas that rapid progress has been achieved over the past ten years.

In the field of optical imaging, there are a number of subsurface imaging techniques currently under development and clinical testing (optical coherence tomography¹, linear and nonlinear emission imaging², confocal microscopy³, harmonic generation imaging⁴). These techniques provide high resolution images of subsurface structures with the drawback that the imaging depth is very small, 1 millimeter or less. This problem is caused by the fact that the image arises from photons that have undergone no scattering until they reach their target. As the imaging depth increases, the number of photons that reach the specific depth without scattering decreases exponentially.

The spectral polarization difference imaging technique (SPDI) was recently proposed as an imaging tool that can provide subsurface imaging at larger depth at the expense of image resolution.^{5,6} This technique utilizes the wavelength dependence of the mean visit depth (\bar{z}) of photons inside a tissue sample before they emerge in the backscattering direction⁷ and polarization means to discriminate against specularly reflected photons at the superficial tissue layers under polarized illumination. The differences in the absorption and/or scattering characteristics of subsurface tissue components are used to highlight their presence at different depth zones by selecting the appropriate illuminating wavelengths. It was proposed that, imaging of structures at different depths inside the tissue can be achieved using the following steps:

- 1) Illuminate the sample with polarized light at different wavelengths (λ_1, λ_2) in order to reach different mean photon penetration depths;
- 2) Record the perpendicular images in order to use the depolarized image component which contains predominantly subsurface image information;
- 3) Adjust the times of exposure $t(\lambda_i)$ to record the images using a normalization factor $N = t(\lambda_1)/t(\lambda_2)$ that allows for normalization of the volume of information (number of photons recorded by the CCD) arising from photons that penetrated only the outer layers of the sample;
- 4) Subtract the perpendicular images obtained under different illuminating wavelengths to obtain a new image containing structures underneath the surface.

The normalization factor described in step (3) is used so that the image information from the outside layers of the tissue is normalized to approximately equal intensity for both illuminating wavelengths. The normalization factor in the first demonstration of the technique was obtained from the ratio of the intensity of the backscattered perpendicular polarization components under 500-fs pulsed illumination and 590-640 nm illumination wavelengths.^{5,6} This work has demonstrated imaging of an "interesting" tissue components located 3.5 mm underneath the surface of the host chicken tissue sample.⁶

In this paper, imaging depth of the order of 1-cm is demonstrated using the spectral polarization difference imaging technique through simplification of the image normalization procedure to avoid the need for time resolved measurements and ultrafast laser illumination. CW, NIR illumination sources are utilized to obtain larger photon mean penetration depths and

achieve deeper subsurface imaging. The illumination is derived from diode lasers or from a white light source in combination with selection of the appropriate wavelength using band-pass optical filters.

2. EXPERIMENTAL SET-UP

The experimental setup is shown in fig. 1. The imaging system involves CW low power illumination of the sample derived from two different illumination assemblies. In the first assembly, the illumination is derived from diode lasers while

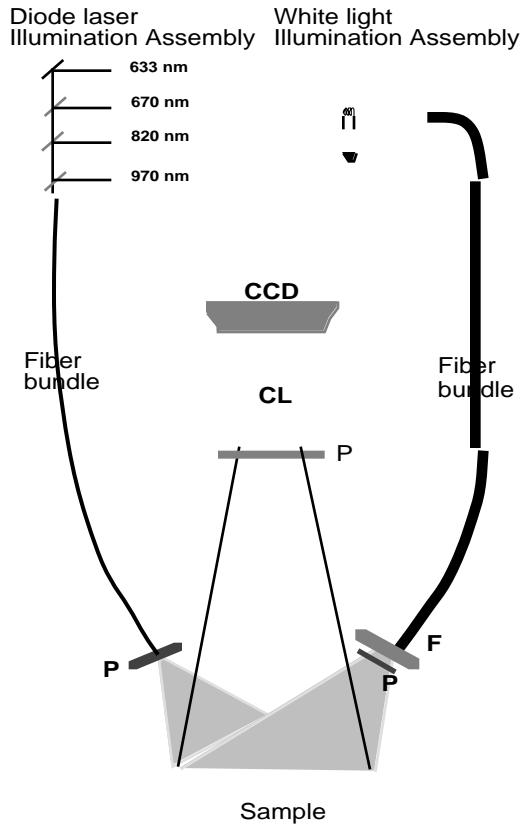


Figure 1. Schematic diagram of the experimental setup of the spectral polarization difference imaging system. The illumination assembly involves diode lasers (left) or a white light source (right). The illumination is delivered into the sample using fibers. Inset shows the cross section of the sample used to demonstrate 1-cm depth subsurface imaging.

in the second assembly, from a white light source.

The illumination assembly that utilizes inexpensive 3 mW output power diode lasers operating at 633 nm, 670 nm, 820 nm and 970 nm is shown on the inset at the left hand side of fig. 1. The output laser beam from each laser is coupled into the input of a fiber using appropriate optics and movable mirrors. A polarizer (P) located at the output of the fiber ensures linearly polarized illumination of the sample. The collection of the backscattered light in the sample is achieved using a 50 mm camera lens (CL). A polarizer (P) in front of the camera lens is used to record only the perpendicular polarization image component using a liquid nitrogen cooled CCD detector. The alternative illumination assembly which utilizes a white light source is shown on the inset at the right hand side of fig. 1. The white light is coupled into a fiber bundle and delivered into the sample. Using appropriate optical filters (F) at the output of the fiber, illumination of the sample with light at different wavelength is achieved. These filters may be narrow band interference filters, relatively broad spectral band filters or, long-pass interference filters. A polarizer (P) positioned after the optical filters ensures polarized illumination of the sample. The image collection assembly is the same as the one described before.

The sample to demonstrate the subsurface imaging technique was prepared using breast chicken tissue as a model medium. A cross section of the sample is shown in fig. 1. In the middle of the front surface of a $5 \times 4 \times 1 \text{ cm}^3$ (1-cm thick) breast chicken tissue layer there was positioned a 4-mm diameter, 1-mm thick fragment taken from a high light scattering material that was used as the “interesting” object to be imaged. Another 1-cm thick layer of breast chicken tissue was then positioned on the top of the object containing breast chicken layer so that the object was sandwiched between the two thick breast tissue layers. The sample was placed between two glass plates and was slightly compressed to a uniform thickness.

3. RESULTS

The perpendicular polarization images of the sample were recorded using the white light illumination assembly and four narrow-band interference filters to select monochromatic illumination of the sample at 600 nm, 690 nm, 770 nm and, 970 nm. The exposure time of the CCD camera to record the image for each illumination wavelength was adjusted so that the digitized image intensity at an arbitrary point at the lower-right part of the image was approximately the same. The images were then subtracted (a second image recorded using a shorter illumination wavelength from a first image recorded using a longer illumination wavelength) to obtain a set of new images. Fig. 2a shows the spectral polarization difference image obtained following subtraction of the 970 nm image from the 600 nm image. Similarly, figs. 2b, 2c and, 2d show the SPDI images obtained from the subtraction of the 970 nm image from the 770 nm image, the 770 nm image from the 690 nm image, the 690 nm image from the 600 nm image, respectively.

The object is viewed with maximum contrast in the images shown in figs. 2a and 2b where the 970 nm illumination was used to record the first direct image. This is due to the maximum photon penetration depth achieved for the longest in wavelength illumination. The object is also visible in the image shown in fig. 2c where the 770 nm illumination was used to record the first direct image. Fig. 2d indicates that when shorter illumination wavelengths are used to obtain the first direct image, the object is no longer visible because the backscattered photons can not reached the object located 1-cm underneath the surface of the tissue to carry its image information.

The experiment was also performed using the diode laser illumination assembly. The results were practically identical. The main difference was that the required exposure time to record a good quality image was of the order of few seconds using

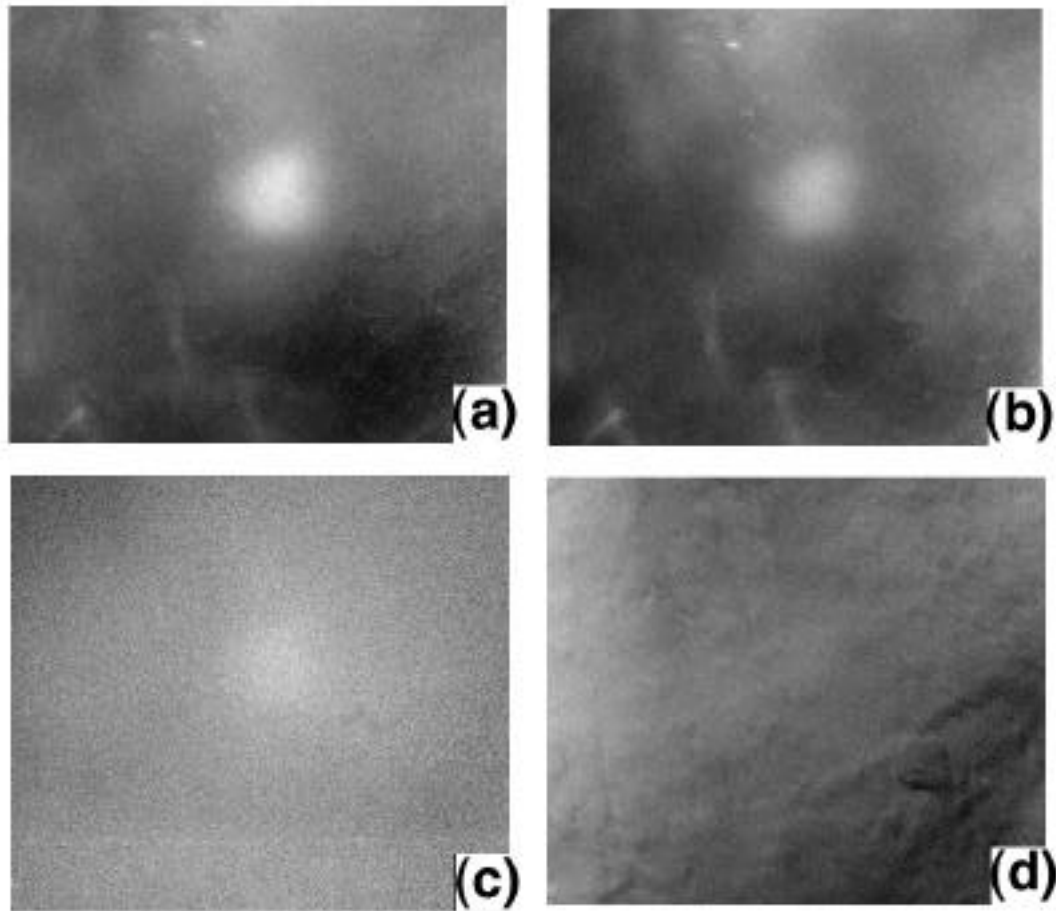


Figure 2: Subsurface images of a high scattering object located 1-cm underneath the surface of breast chicken tissue obtained using the spectral polarization difference imaging technique and 600 nm, 690 nm, 770 nm and, 970 nm illumination. a) [970-600] nm SPDI image, b) [970-770] nm SPDI image, c) [770-690] nm SPDI image and, d) [690-600] nm SPDI image.

the white light illumination assembly and of the order of 100 msec. using the diode laser illumination assembly.

The ability of the technique to image tissue structures at different depth zones is best demonstrated in fig.3. The white light illumination assembly with subsequent wavelength selection using four different optical filters was employed to image the veins of the arm of a human male having well developed muscle structure and deep below surface veins. The optical filters utilized were: 850 nm long pass (Image # Im850LP), 690 nm narrow band (Image # Im690NB), 640 nm narrow band (Image # Im640NB), and 600 nm narrow band (Image # Im600NB). Fig 3a shows the SPDI subsurface image obtained using images Im850LP and Im600NB. The large difference in the illumination wavelength leads to a wider imaging depth zone which in this image reveals the presence of three vein branches denoted with the numbers 1 through 3. Fig 3b shows the SPDI image obtained using the images Im690NB and Im600NB. The shorter illumination wavelengths and smaller difference in wavelength lead to a smaller imaging depth and narrower imaging depth zone than in the previous case. The result is that only the vein branch “3” is visible in this image arising from the fact that it is located closer to the surface (skin). Fig. 3c shows the SPDI subsurface image obtained using images Im850LP and Im690NB. The longer illumination wavelengths and their smaller difference in wavelength lead to a larger penetration depth and imaging at an inner depth zone. The result is that this image contains only the vein branches “1” and “2” which are located deeper underneath the surface while the vein branch “3” (for which image 3b indicated that is located closer to the surface) is not visible. In addition, a new branch “4” is also visible. Fig. 3d shows the SPDI image obtained using images Im690NB and Im640NB. The smaller difference in the illumination wavelengths leads to an image that contains no information regarding the vein structure. From the vein branches shown in the images of figs. 3a, 3b and 3c, only branch “3” is barely visible to the naked eye under white light illumination.

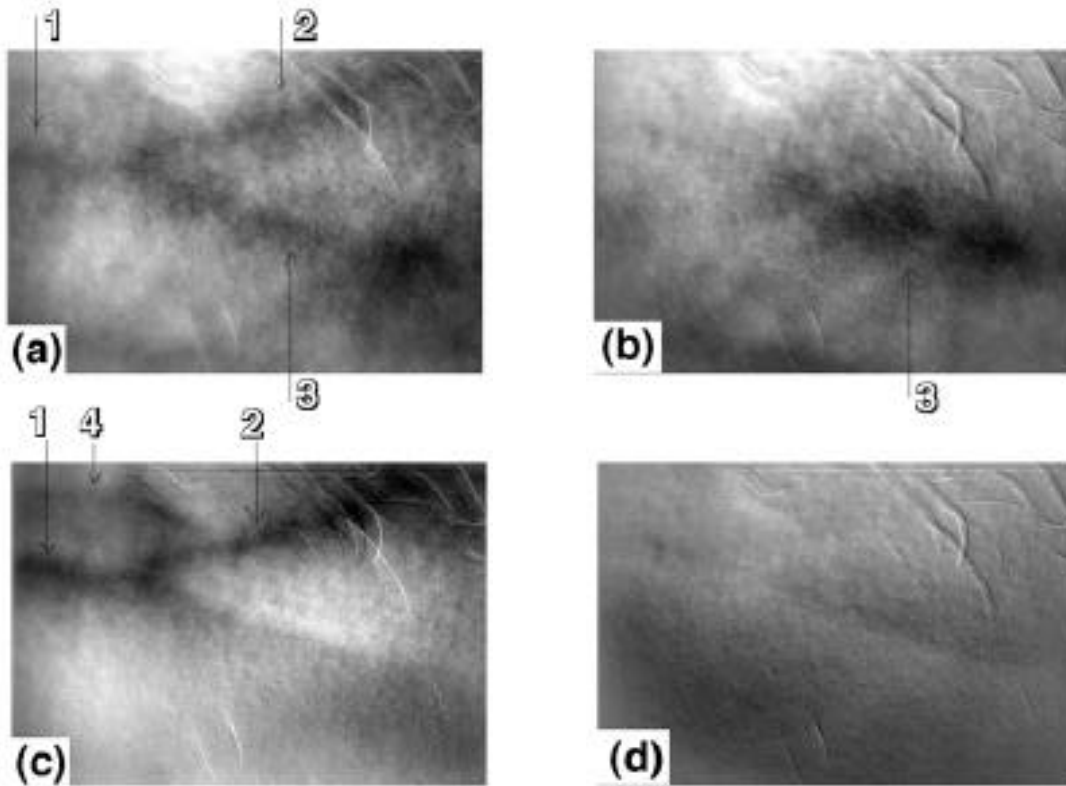


Figure 3: Subsurface SPDI images of veins of the arm of a human male obtained using illumination spectral bands λ_1 and λ_2 obtained from a white light source and spectral filtering using the following optical filters: a) $\lambda_1 = 850$ nm long pass and $\lambda_2 = 600$ nm narrow band, b) $\lambda_1 = 690$ nm narrow band and $\lambda_2 = 600$ nm narrow band, c) $\lambda_1 = 850$ nm long pass and $\lambda_2 = 690$ nm narrow band, d) $\lambda_1 = 690$ nm narrow band and $\lambda_2 = 640$ nm narrow band.

4. DISCUSSION

The experimental results shown in figs 2, and 3 demonstrate the ability of the technique to acquire deep-subsurface images in tissues. The utilization of NIR illumination allows a sufficient portion of the backscattered photons to reach large photon penetration depths and carry through the image information of on object located underneath the surface. The main

difference of the present work when compared to the original version of the technique^{5,6} is the simplification of the normalization procedure to acquire the image. This was achieved by adjusting the exposure time of the CCD camera to record the image for each illumination wavelength so that the digitized image intensity at an arbitrary point of the image was approximately the same. This simplification of the normalization procedure makes the utilization of inexpensive CW light sources possible and eliminates the need for ultrashort tunable laser and time resolved measurements without affecting the image quality. This improvement has made the technique more suitable for a clinically relevant environment.

The subsurface spectral polarization difference imaging technique described in this work based on subtraction of the perpendicularly polarized images obtained under different wavelengths can be used to highlight differences in absorption by blood from arteries and veins or scattering due to the presence of different types of tissue at different depths zones inside a host tissue.^{5,6} A structure inside the tissue that exhibits higher absorption will emerge in the polarization difference image darker in intensity as shown in fig. 3. A structure that scatters and depolarizes light more will appear in the polarization difference image brighter than the rest of the image as demonstrated in fig. 2. It has been reported that human breast tumor tissues scatter and depolarize light more than normal tissue.⁸ Therefore, the subsurface imaging technique described here may be also very useful in detecting tumor lesions underneath the surface of a normal tissue using tunable NIR light such as from 800 to 1300 nm.

Utilization of different illuminating wavelengths to record the backscattered images of a tissue sample provides a set of images where the mean photon penetration depth gradually changes. The normalization procedure and the fact that the perpendicular polarization image component contains no specular reflections from the surface of the tissue lead to the removal of the image component of the outer layers of the tissue during image subtraction. With gradual change of the illuminating wavelengths one can effectively image structures at different depth zones depending on the photon penetration depth of the pair of illumination wavelengths utilized to record the original images. A large difference in the two wavelengths gives rise to a wider depth zone as shown in fig. 3a. A smaller difference in the illuminating wavelengths leads to narrower depth zones as shown in figs. 3b and 3c. The maximum imaging depth depends on the scattering and absorption characteristics of the tissue sample and the illumination wavelengths. Fig. 2 shows that the 970 nm photons can reach (and consequently be used to image) the object located 1 cm underneath the surface of the host breast chicken tissue. Using the 770 nm illumination in the same experiment, the image quality degrades due to the fact that the 770 nm photons barely reach the 1 cm depth before backscattering. In another experiment, the same object was position 1.5 cm underneath the surface of the tissue and imaging was only possible using the 970 nm illumination.

5. CONCLUSION.

The spectral polarization difference imaging technique can be used to obtain subsurface images of “interesting” objects at different depth zones and as deep as 1-cm underneath the surface. Images underneath the surface of the host tissue can be obtain by subtraction of the perpendicular polarization components at different illuminating wavelengths. This technique is ideal for highlighting the presence of veins or vessels as well as tissue lesions having different NIR light scattering characteristics.

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